DNA Microarrays Using Magnetic Labeling and Detection

There are myriad applications for biosensors—from antiterrorism, to food safety, to point-of-care clinical diagnostics. The goal is to create systems that mimic the sensitive and specific sensing abilities that come effortlessly to naturally occurring organisms. A common approach to detecting biological molecules is to attach to the target molecule a chemical label that produces an externally observable signal. Traditionally, this is accomplished using biomolecular recognition between the target molecule and a specific receptor (e.g., an antibody) that incorporates a label such as a radioisotope, enzyme, or fluorescent molecule. Sensing methods have been developed based on a range of transduction mechanisms, including optical, electrical, electrochemical, thermal, and piezoelectrical means (see *Biomedical Sensors: Materials*), which are covered eloquently by numerous reviews (see, e.g., Pearson *et al.* 2000, Vo-Dinh and Cullum 2000).

Recently, magnetic particles have been developed as labels for biosensing. Magnetic labels have several potential advantages over other labels. They are not subject to degradation over time or photobleaching, making their properties very stable. In addition, magnetic fields are not attenuated by biological material, offering the possibility of *in vivo* sensing. Magnetism may also be used to remotely manipulate the particles. Finally, there are a number of sensitive magnetic field detection methods arising from advances in microelectronics and magnetic storage technologies that will enable the design of compact, low-power sensor systems. Recently described detection methods include the Maxwell bridge (Kriz *et al.* 1996), micro-cantilever-based force amplified biological sensor (Baselt *et al.* 1997), superconducting quantum interference device (SQUID) (Kötitz *et al.* 1997), giant magnetoresistive bead array counter (Baselt *et al.* 1998), frequency-dependent magnetometer (Richardson *et al.* 2001), and silicon Hall sensor (Besse *et al.* 2002).

1. Basic Biomolecule Labeling and Detection with Magnetic Particles

As for many other biomolecular labels, magnetic labeling can be easily accomplished using specific ligand–receptor interactions, as illustrated generically in Fig. 1. The illustrated "sandwich" configuration proceeds as follows:

(i) Receptor molecules specific for the target biomolecules are attached to the surface of a solid substrate. Often arrays of different probe spots are used to simultaneously detect multiple targets.

(ii) When target molecules are present in a sample solution, they are captured by the surface.

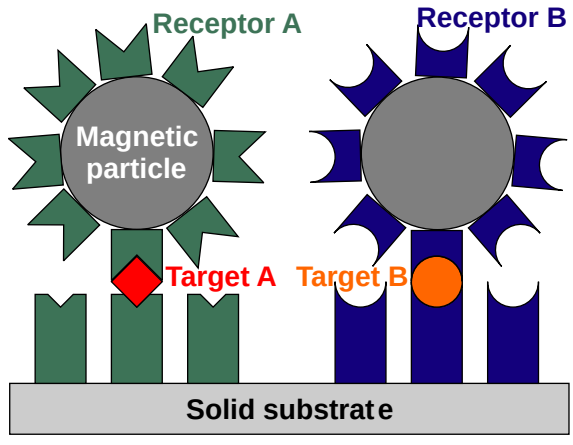

Figure 1
Generic illustration of magnetic labeling of targets captured onto a solid substrate in a "sandwich" configuration using specific biomolecular ligand–receptor recognition.

(iii) Magnetic particles coated with a second set of receptor molecules for the target are introduced, labeling the previously captured targets.

(iv) The label particles are detected by a magnetic sensor.

Sandwich assays are commonly performed using antibody–antigen pairs and complementary DNA strands, and often use the strongly binding biomolecular combination of streptavidin and biotin to attach the particle labels to the receptor molecules.

For biosensing applications, the magnetic particles used as labels should be paramagnetic, i.e., only have a magnetic moment when a magnetic field is applied. The absence of a permanent magnetic moment prevents agglomeration of the particles in solution which would render them ineffective as labels. When selecting label particles, properties of importance include the size and size uniformity, the type of magnetic material and its volume fraction within the particle, and the particle surface chemistry. For example, though nanometer-scale particles are matched in size to biological macromolecules such as DNA, the small magnetic fields they generate are difficult to detect. Second, the surface of the particles must be suitable for chemical attachment of biomolecules. A variety of paramagnetic beads with biocompatible polymer or silica shells are commercially available with diameters that range from 1 μm to 5 μm.

Because magnetic particles experience a force in the presence of a magnetic field gradient, they may be manipulated by magnets. This phenomenon can be used to apply a controlled force that selectively pulls off only those labels not bound to the surface by specific binding (Lee *et al.* 2000). Such a force

discrimination assay increases the sensitivity of detection by greatly reducing the background signal and thereby permitting very low signal levels to be detected with confidence.

2. Magnetic Particle Detection

The detection of magnetic particles can be accomplished by a number of methods, as mentioned above. Here we will focus on the use of solid-state magnetic field sensors embedded in the substrate, a method that illustrates many of the advantages of magnetic labeling for biomolecule detection. For example, such an approach has the potential to eliminate the requirement for an external detection system, thereby reducing the size, cost, and power of the sensor system.

One of the simplest solid-state magnetic field sensors to incorporate into a microelectronic substrate is a giant magnetoresistive (GMR) wire (Baselt *et al*. 1998). GMR materials are multi-component thin films with alternating magnetic and nonmagnetic layers (see *Giant Magnetoresistance*). The relative orientation of the magnetic moments of these layers is altered in the presence of a magnetic field, changing the electrical resistance of the wire. When a magnetic particle is present above a GMR sensor, the resistance of the sensor changes; the larger the number of particles present, the larger the change. The availability of suitably sensitive GMR sensors was made possible by recent advances in magnetic materials for high-density data storage, including magnetic disk drive read-out sensors and nonvolatile magnetic memory elements (Prinz 1998).

When paramagnetic microbeads are used, such as those commercially available, GMR sensors have been designed that are only sensitive to a planar component of the dipolar magnetic field induced in the particles, as illustrated in Fig. 2. As discussed by Miller *et al*. (2001), when an external magnetic field is applied normal to the plane of the GMR sensor, the field strength at the sensor is given by

$$B \sim m/z^3 \tag{1}$$

where m is the magnetic moment of the bead and z is the distance from the center of the bead to the sensor. Therefore, the resistance change in the sensing wire is roughly proportional to the number of magnetic particles present above it, a signal that can be easily measured. For maximum signal, the particle should have as large a magnetization as possible and be as close to the sensor as possible.

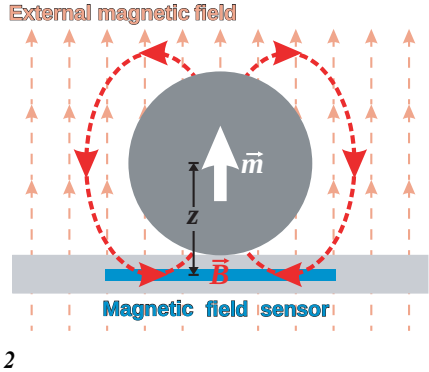

Figure 2
Schematic of the magnetic field induced around a paramagnetic microbead on top of a magnetic field sensor when an external field is applied normal to the substrate.

3. Detection with a GMR Sensor Microarray

A well-developed system that uses a GMR microarray to detect different DNA sequences in a sample is the bead array counter (BARC) sensor (Baselt *et al*. 1998, Edelstein *et al*. 2000, Miller *et al*. 2001). In this system, the test is performed inside a small, quartz flow cell mounted over the sensor chip. The chip itself is wire bonded to a printed circuit board housed in a disposable plastic cartridge that contains the required liquid reagents. The cartridge plugs into an automated electronic controller and connects to a miniature pumping system (Tamanaha *et al*. 2002).

Before assembly, the surface of the sensor chip is coated with a biocompatible polymer film (polyethylene glycol (PEG)) and an array of single-stranded DNA capture probes (Fig. 3(a)). Note that the GMR sensors and associated interconnects must be protected from the reactive and conductive salt solution used in the biochemical test, in this case by a film of silicon nitride. The chip is also coated with a thin gold layer so that the DNA probes and PEG can be attached to the surface by strong gold–sulfur bonds. Each sensor zone is covered with strands of DNA complementary for a different target strand. The PEG film inhibits undesirable, nonspecific, sticking of target DNA and magnetic particles in the areas outside of the DNA probe spots.

To test for the presence of specific target DNA sequences, the sample is introduced into the flow cell and allowed to hybridize with the capture probes. In the illustrated example, the target strands have been pre-modified to have biotin molecules attached at one end. Therefore, the presence of target in the sample will result in double-stranded, biotin-tagged DNA bound above a sensor. The captured targets are then labeled with streptavidin-coated magnetic microbeads. Beads just resting on the surface can be removed by a magnetic force, and the remaining beads detected by the underlying GMR sensors.

The results of an actual experiment performed in this way are shown in Fig. 3(b) on a chip with eight sensor zones (Miller *et al*. 2001). A micrograph shows

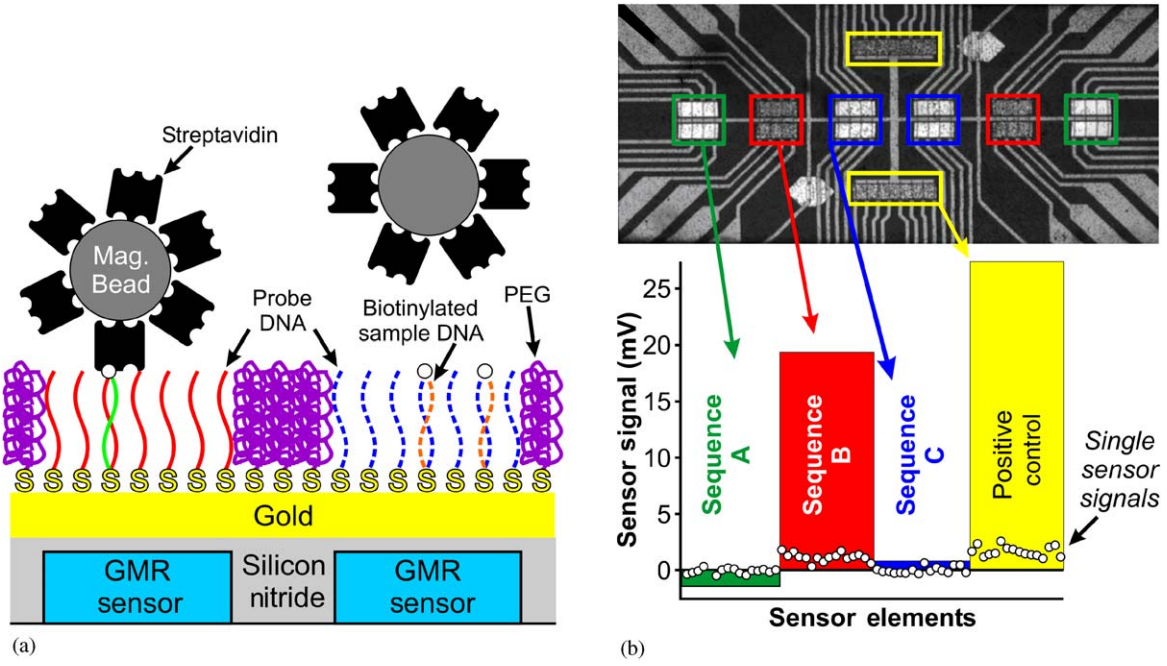

Figure 3

(a) An illustration of the BARC biosensor approach. Note that the elements are not to scale; in particular, the beads and sensors are much larger in proportion to the biomolecules. (b) A micrograph of a BARC chip after a DNA test, with the eight sensing zones outlined. For each of the probe zones, the graph shows the measured signals from the individual sensing elements (open circles) and the integrated signal for all 16 elements (colored bar). The assay for sequence "B" was performed with 10 nM DNA hybridized for 30 min. The total test required $\sim$60 min.

the surface of the chip after the test. In this test, each of the three different DNA sequences and the positive control (a probe with biotin already on it) were each applied to two zones. Introducing a sample containing only sequence "B" results in the complementary zones and the positive control being covered with beads (appearing dark). The presence of the beads is accurately detected by the eight GMR sensors in each zone.

4. Summary

Magnetic labeling and detection holds great promise for sensing biomolecules. As demonstrated by the BARC system, advances in microelectronics and materials can be adapted to analytical applications in the biosciences with great success. Magnetic particles, magnetic force manipulation, and magnetoelectronic detection can be combined to create relatively simple, compact, sensor systems. Such technology promises to fulfill the need for ever faster, more sensitive, and more portable systems in fields as diverse as homeland defense, clinical diagnostics, genomics, proteomics, and forensics.

Bibliography

Baselt D R, Lee G U, Hansen K M, Chrisey L A, Colton R J 1997 A high-sensitivity micromachined biosensor. *Proc. IEEE* **85**, 672–80

Baselt D R, Lee G U, Natesan M, Metzger S W, Sheehan P E, Colton R J 1998 A biosensor based on magnetoresistance technology. *Biosens. Bioelectron.* **13**, 731–9

Besse P-A, Boero G, Demierre M, Pott V, Popovic R 2002 Detection of a single magnetic microbead using a miniaturized silicon hall sensor. *Appl. Phys. Lett.* **80**, 4199–201

Edelstein R L, Tamanaha C R, Sheehan P E, Miller M M, Baselt D R, Whitman L J, Colton R J 2000 The BARC biosensor applied to the detection of biological warfare agents. *Biosens. Bioelectron.* **14**, 805–13

Kötitz R, Matz H, Trahms L, Koch H, Weitschies W, Rheinländer T, Semmler W, Bunte T 1997 SQUID based remanence measurements for immunoassays. *IEEE Trans. Appl. Supercond.* **7**, 3678–81

Kriz C B, Rådevik K, Kriz D 1996 Magnetic permeability measurements in bioanalysis and biosensors. *Anal. Chem.* **68**, 1966–70

Lee G U, Metzger S, Natesan M, Yanavich C, Dufrêne Y F 2000 Implementation of force differentiation in the immunoassay. *Anal. Biochem.* **287**, 261–71

Miller M M, Sheehan P E, Edelstein R L, Tamanaha C R, Zhong L, Bounnak S, Whitman L J, Colton R J 2001

A DNA array sensor utilizing magnetic microbeads and magnetoelectronic detection. *J. Magn. Magn. Mater.* **225**, 138–44

Pearson J E, Gill A, Vadgama P 2000 Analytical aspects of biosensors. *Ann. Clin. Biochem.* **37**, 119–45

Prinz G 1998 Magnetoelectronics. *Science* **282**, 1660–3

Richardson J, Hawkins P, Luxton R 2001 The use of coated paramagnetic particles as a physical label in a magneto-immunoassay. *Biosens. Bioelectron.* **16**, 989–93

Tamanaha C R, Whitman L J, Colton R J 2002 Hybrid macro–micro fluidics system for a chip-based biosensor. *J. Micromech. Microeng.* **12**, N7–17

Vo-Dinh T, Cullum B 2000 Biosensors and biochips: advances in biological and medical diagnostics. *Fresenius J. Anal. Chem.* **366**, 540–51

Cy R. Tamanaha and Lloyd J. Whitman

Encyclopedia of Materials: Science and Technology
ISBN: 0-08-043152-6
pp. 1–4